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INTERNATIONAL SEARCHING AUTHORITY

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PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)	
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below
International application No. PCT/EP2005/002870	International filing date *(day/month/year)* 17.03.2005
Priority date *(day/month/year)* 25.03.2004	
International Patent Classification (IPC) or both national classification and IPC C12Q1/68	
Applicant GLOBAL GENOMICS AB	

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2005/002870

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority for the purposes of search.

3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43*bis*.1 and 64.1) is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	9-11, 15-17, 27-31 and 45
	No:	Claims	1-8, 12-14, 18-26 and 32-44
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-45
Industrial applicability (IA)	Yes:	Claims	1-45
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

V. Reasoned statement (Continuation)

2.1 CITATIONS:

Reference is made to the following documents:

D1: US-B1-6 316 229 (LIZARDI PAUL M ET AL) 13 November 2001 (2001-11-13)

D2: US-B1-6 274 320 (ROTHBERG JONATHAN M ET AL) 14 August 2001 (2001-08-14)

D3: US 2003/054396 A1 (WEINER MICHAEL P) 20 March 2003 (2003-03-20)

D4: HATCH A ET AL: "Rolling circle amplification of DNA immobilized on solid surfaces and its application to multiplex mutation detection" GENETIC ANALYSIS: BIOMOLECULAR ENGINEERING, ELSEVIER SCIENCE PUBLISHING, US, vol. 15, no. 2, April 1999 (1999-04), pages 35-40, XP004223009 ISSN: 1050-3862

D5: WO 90/04652 A (DNAX RESEARCH INSTITUTE OF MOLECULAR AND CELLULAR) 3 May 1990 (1990-05-03)

D6: US-B1-6 270 961 (DRMANAC RADOJE) 7 August 2001 (2001-08-07)

D7: US 2003/036084 A1 (HAUSER BRIAN ET AL) 20 February 2003 (2003-02-20)

D8: MIRZABEKOV A D: "DNA SEQUENCING BY HYBRIDIZATION -A MEGASEQUENCING METHOD AND A DIAGNOSTIC TOOL?" TRENDS IN BIOTECHNOLOGY, ELSEVIER, AMSTERDAM,, GB, vol. 12, no. 1, January 1994 (1994-01), pages 27-32, XP000670232 ISSN: 0167-7799

2.2 NOVELTY (Art. 33(2) PCT)

2.2.1 D1 discloses a nucleic acid sequencing method (or re-sequencing, detection of SNPs; see Abstract, last sentence; column 66, lines 40-44) which comprises:

(i) providing a DNA sample containing a plurality of circular single-stranded DNA template molecules, each comprising a target sequence and a primer annealing

sequence (column 4, lines 50-65; column 44, line 21, to column 45, line 14);

(ii) forming an array of immobilized and amplified template molecules by rolling-circle amplification of the template using an immobilized primer (column 5, lines 12-15; column 19, lines 25-41; column 34, lines 22-67); although a table of correspondence between primer sequences and array locations is known (column 33, lines 9-15), this array falls within the unclear boundaries of the term "random" (see below, lack of clarity) because D1 does not describe any specific criteria for distribution of primers over the array, and therefore they are considered to have been arbitrarily distributed;

(iii) probing the tandem-repeated amplification product with a panel of probes and obtaining a hybridization spectrum of the target (column 24, lines 42-53; column 48, lines 39-67);

(iv) comparing the hybridization spectrum to a hybridization spectrum for reference sequences; this general step is implicit to microarray technology as used in D1 (column 32, lines 36-46).

Hence, despite the fact that the methods described in the present specification, especially pages 10-27, differ substantially from the methods of D1, the subject-matter of claim 1 in its present wording is not new (see also below, lack of essential features).

2.2.2 The array of claim 32 is anticipated by the following prior art passages:

D1: see above mentioned passages; see also column 19, lines 25-41;

D2: Figure 1; columns 10-11; column 7, lines 8-10;

D3: paragraphs 18-22 and 103;

D4: Abstract; Figure 1;

It must be noted that the feature reciting that "each sequence represents a *random* fragment from an initial [...] library" does not introduce any effective limitation to the claimed product. Even if the origin of the nucleic acid fragments were clearly specified, it is not apparent which technical features would it confer to the fragments as such to make them distinguishable from the prior art.

Hence, the subject-matter of claim 32 is not new.

2.2.3 The set of probes of claim 39 is anticipated by the following prior art passages:

D1: see above mentioned passages; see also column 24, lines 42-53;

D5: Abstract; Figure 1; page 3, paragraph 3, to page 10, paragraph 2;

D6: Abstract; column 2, lines 50-56; col. 3, lines 15-21; Examples 1, 15 and 18;

D7: Examples 1-4;

D8: pages 27-28.

Again, it must be noted that the feature reciting "is such that at least 10% of all positions in a random or arbitrary target sequence statistically hybridize with at least one probe in the set of probes" does not introduce any effective limitation, not only because of the lack of clarity of the desideratum feature (see below), but also because since the arbitrary target sequence is not part of the claimed product, it will always be possible to define a sequence which fulfills said criteria.

2.2.4 Therefore, the present application does not satisfy the criterion set forth in Article 33(2) PCT because the subject-matter of claims 1-8, 12-14, 18-26 and 32-44 is not new in respect of prior art as defined in the regulations (Rule 64(1)-(3) PCT).

2.3 INVENTIVE STEP (Art. 33(3) PCT)

2.3.1 Claims 9-11, 15-17, 27-31 and 45 do not appear to contain any additional features which, in combination with the features of any claim to which they refer, involve an inventive step, because they fall under the scope of routine experimental design and optimization, and the skilled person would include these features in order to solve the problem posed without the involvement of an inventive step.

2.3.2 Therefore, the present application does not satisfy the criterion set forth in Article 33(3) PCT because the subject-matter of claims 1-45 does not involve an inventive step (Rule 65(1)(2) PCT).

2.3.3 Should the **essential features** listed below be explicitly recited to limit the method of claim 1, the following reasoning would apply:

Document D2 would be considered to represent the most relevant state of the art as it discloses a nucleic acid sequencing method comprising (see Figure 1; column 3, line 17, to column 4, line 40; column 5, line 55, to column 14, line 49):

(i) providing a DNA sample containing a plurality of circular single-stranded DNA template molecules, each comprising a target sequence and a primer annealing sequence;

(ii) forming an array of immobilized and amplified template molecules by rolling-circle amplification of the template using an immobilized primer;

(iii) determining the nucleotide sequence of the amplified product by pyrosequencing.

The subject-matter of said hypothetical claim 1 differs in that the step (iii) of sequence determination is performed using a *sequence-by-hybridization* approach (SBH). The technical effect of this difference is double, a higher throughput of analysed sequences and a longer read length per template.

The problem to be solved by the subject-matter of said hypothetical claim 1 would therefore be regarded as the provision of an improved method for nucleic acid sequencing. The solution would be to modify the method of D2 by performing the sequence determination step using an SBH technique instead of a pyrosequencing technique.

This solution would not however be considered as involving an inventive step because SBH, its methodological steps, tools, advantages and limitations were known and available to the skilled person at the date of priority. This teaching can be found in anyone of documents D5 to D8 (see above mentioned passages).

VIII. Certain Observations (Continuation)

1 The application does not meet the requirements of Article 6 PCT because claims are not clear for the following reasons:

1.1 The term "random" used in claims 1 and 32 does not have a unique meaning in the field, and in the present context it is not clear whether it makes reference to a non-parallel distribution of spots, a distribution of samples regardless their identity, or a

distribution of sample to only some arbitrarily chosen spots.

1.2 The term "effective specificity" used in claims 3, 19, 20, 39 and 40 does not have a clear definite meaning for the skilled person, casting doubts about the difference between *effective* and *ineffective* specificity. The features assigned to this term by the present description, although clear from page 20, lines 24-30, are not self-evident.

2 It appears from the description that the following technical features are essential to the present invention:

a) the RCA primer is **common** to all circular templates, or universal (see page 11, lines 18-29);

b) the sequence determination step is based on a **sequencing-by-hybridization** approach, which implies using a panel of probes representing all possible sequences of a certain length (see page 5, line 24; page 19, line 21, to page 23, line 33).

These essential technical features are however not present in independent claim 1. For these reasons claim 1 lacks clarity according to Art. 6 PCT taken in combination with Rule 6.3 (b) PCT (see also PCT Guidelines 5.33).

3 Claims 5-8 and 39-44 do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The claims attempts to define the subject-matter in terms of the result to be achieved which merely amounts to a statement of the underlying problem: "adjusted so that the statistical probability of hybridization of each probe to each target is between...". The technical features necessary for achieving this result are however missing.